

Toxicogenomics: The Application of Gene Expression in Toxicology Screening

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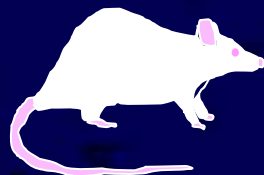
ToxExpress® Program

- Program has been built with guidance of major pharmaceutical companies



- Reference database built with carefully selected and annotated marketed pharmaceuticals, drugs taken off the market and chemicals

Predictive Toxicogenomics



Rat exposed to NCE

General Model

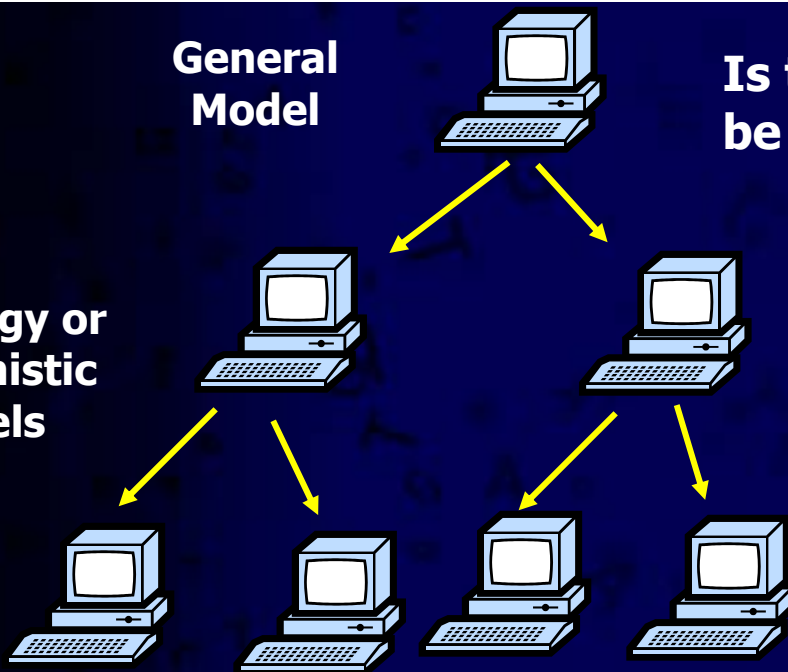
Is the compound likely to be a human toxicant?

Pathology or Mechanistic Models

What type of injury will it induce in humans or rats?

Compounds

What well-known compounds does it resemble?



Validation Methods

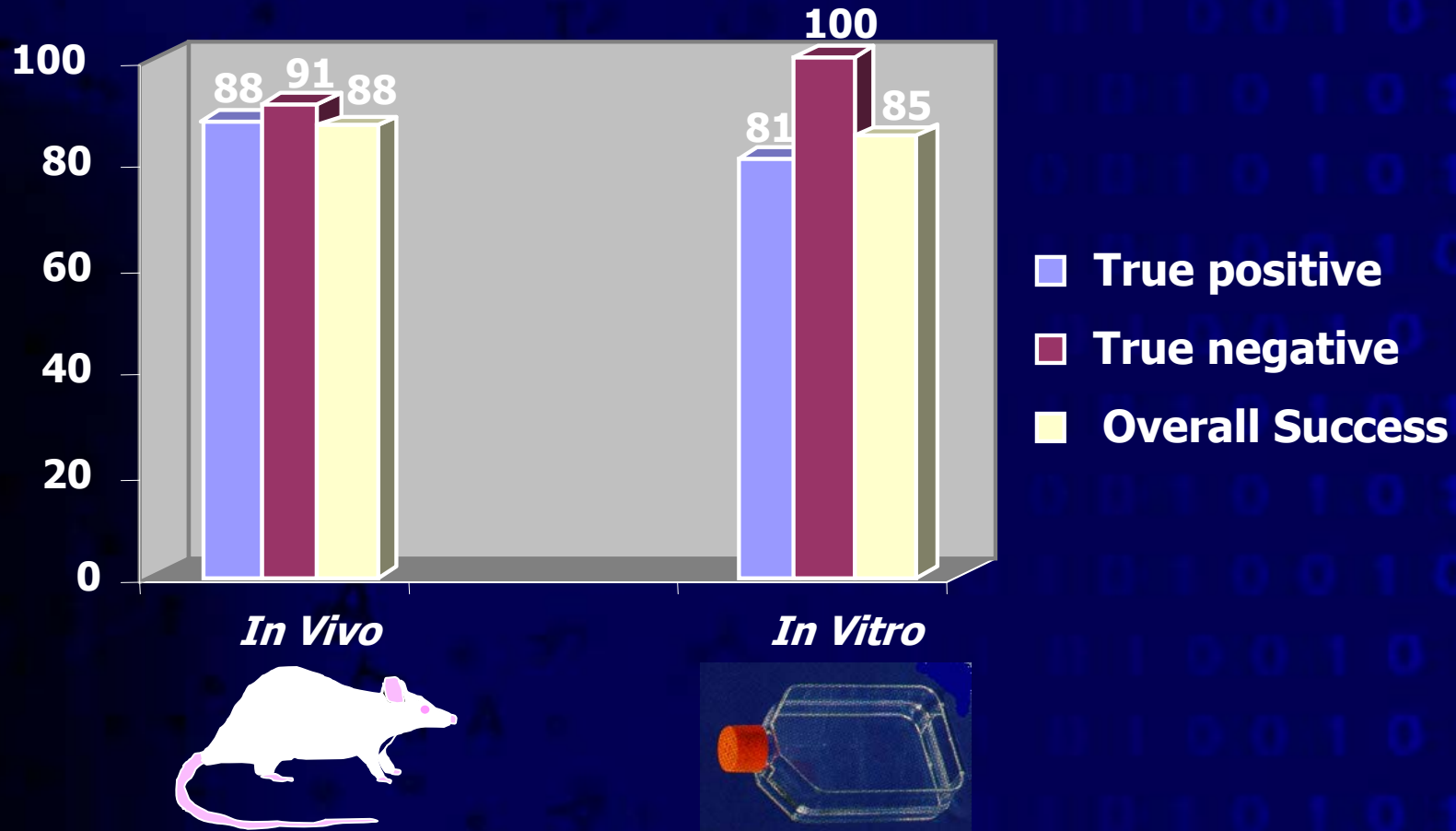
- **Cross-validation**
 - **2/3::1/3 Split**
 - Remove 1/3 of the data and rebuild model
 - **Compound Drop**
 - Remove a single compound's data and rebuild model
 - **Always re-select genes after removal of data**
- **External validation using customer-supplied liver data demonstrates results very similar to statistical validation described above**

General Hepatotoxicity Modeling Results



*** Compound Drop Validation (=Worst Case Scenario)**

General Hepatotoxicity Modeling Results



*** Compound Drop Validation (=Worst Case Scenario)**

Examples Correctly Predicted in Both Systems Using General Toxicity Model

Compound			Action
Flutamide	Toxic	Toxic	Human specific injury
Acetaminophen	Toxic	Toxic	Necrosis
Tamoxifen	Toxic	Toxic	Cholestasis
Tetracycline	Toxic	Toxic	Microvesicular steatosis and hepatitis

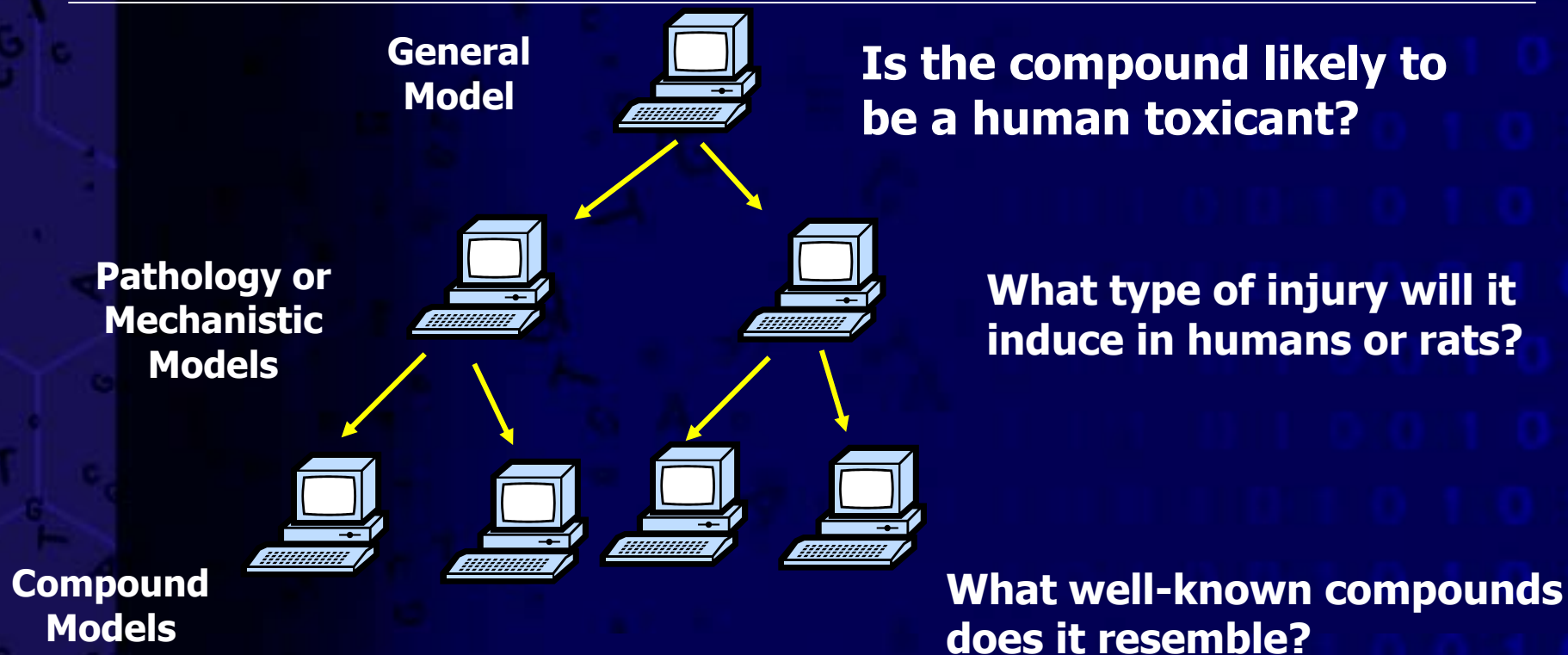
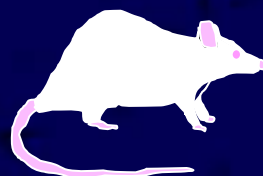
Felbamate

- **Approved in 1993**
- **Success with several intractable seizure diseases, particularly in children**
- **13 deaths due to aplastic anemia**
- **Restricted in 1994 due to hepatotoxicity**
 - **Phase III trial demonstrated increases in AST/ALT in ~3.5% of patients**
 - **5 deaths due to fulminant hepatic failure**
- **Known to cause liver enlargement in rats and hepatic adenomas in female rats and in mice**

Gene Logic's Felbamate Study

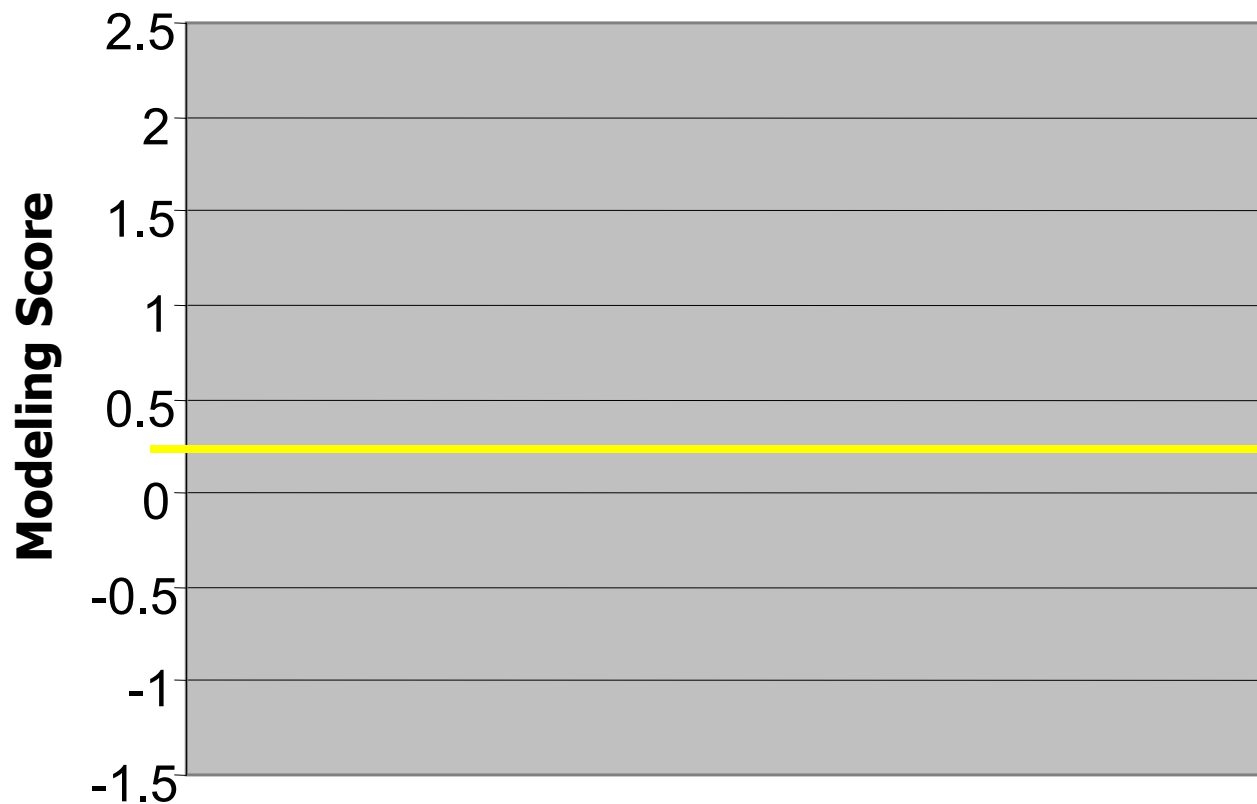
- **Doses administered daily via oral gavage**
- **Rats sacrificed at 6 hr, 24 hr, 336 hr post first dose**
- **Clinical chemistry signs of hepatotoxicity:**
 - 6 and 24 hr: none
 - 336 hr: minor but statistically significant increase in ALT (56%) and ALP (33%)
 - Both within historical normal range
- **Histology:**
 - Liver: Normal at all time points

Predictive Toxicogenomics



General Model Results

Felbamate Predictions



**Ref DB behavior
(1000's of samples)**

Above threshold:
Ref DB
compounds that
cause
hepatotoxicity

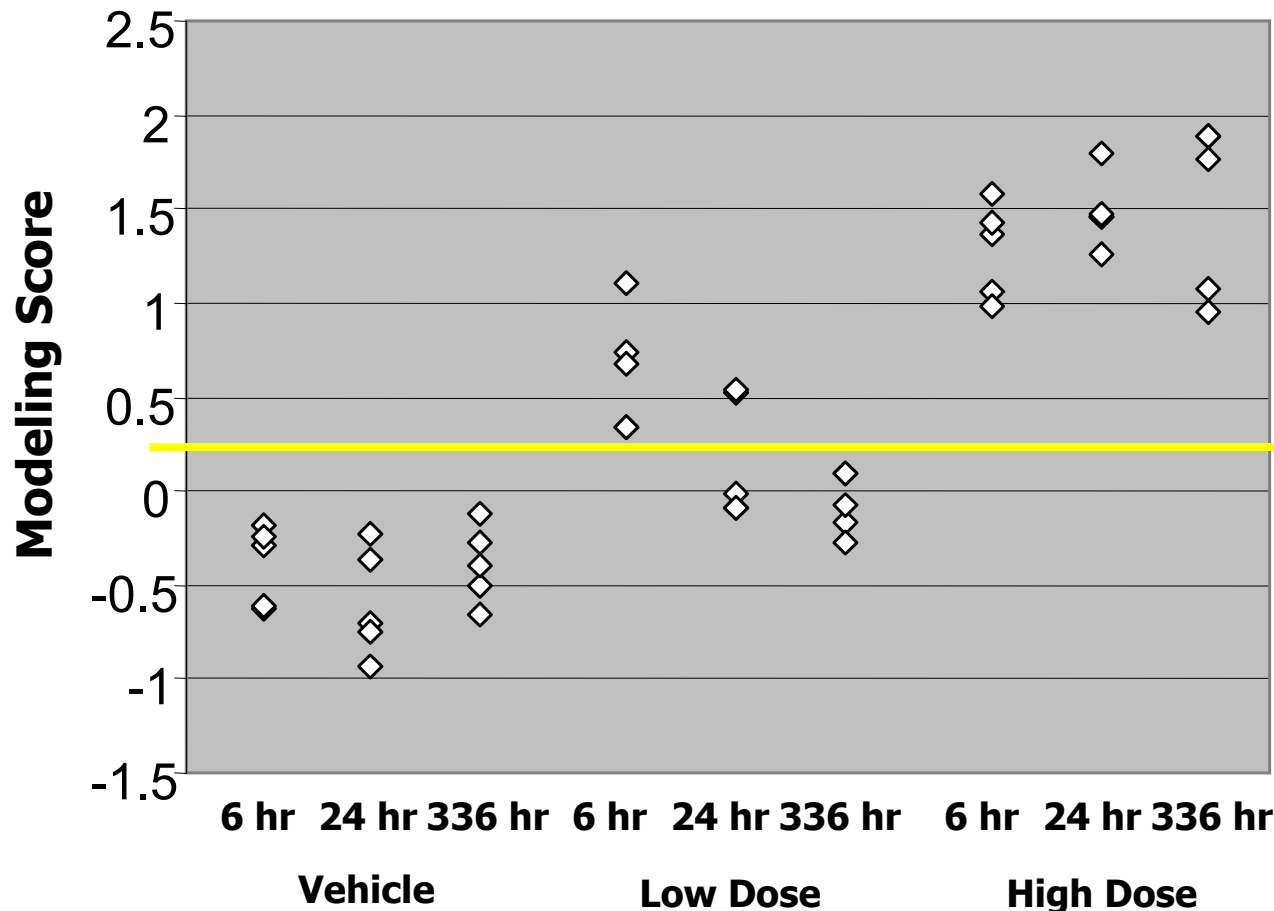
**Validated
Threshold**

Below threshold:
Ref DB vehicles,
compounds that
do not cause
hepatotoxicity

General Model Results

Ref DB behavior
(1000's of samples)

Felbamate Predictions



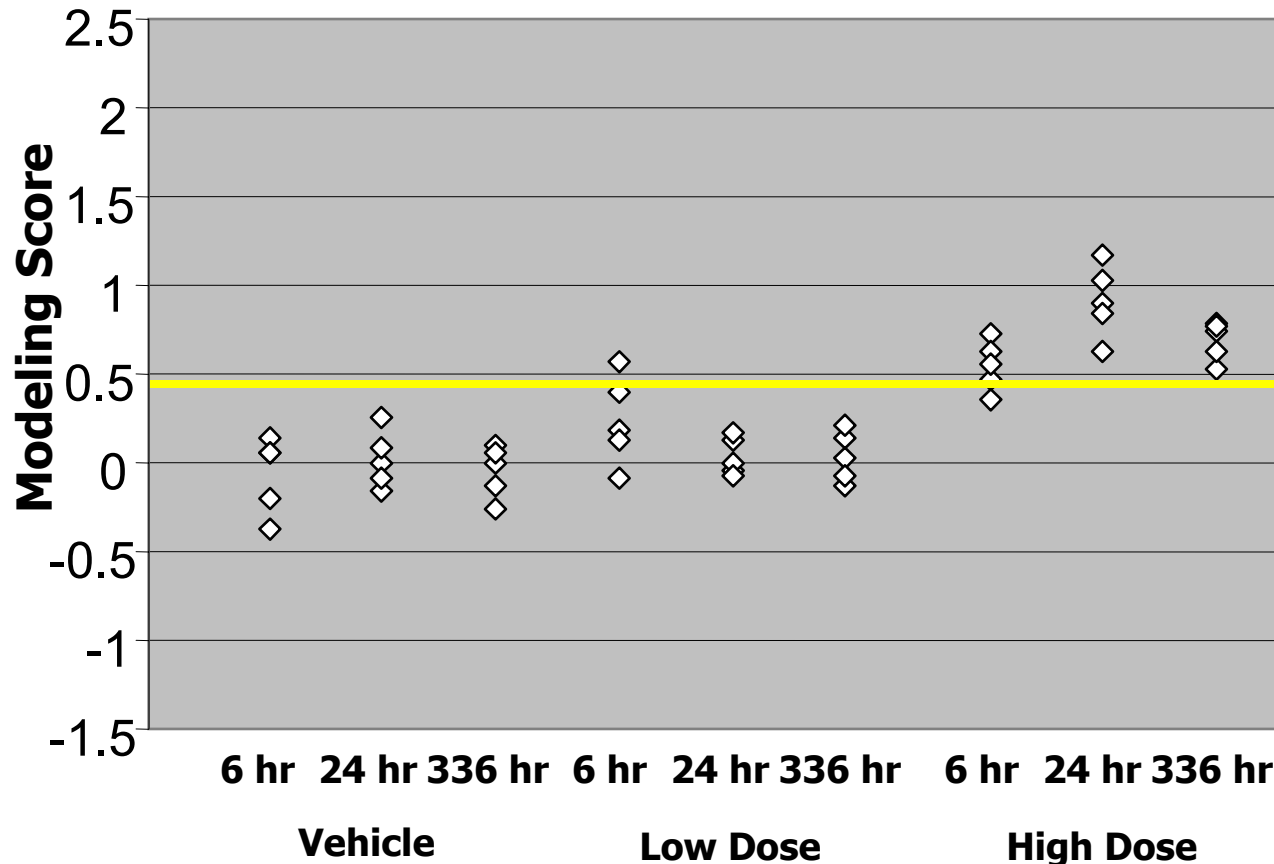
Above threshold:
Ref DB
compounds that
cause
hepatotoxicity

**Validated
Threshold**

Below threshold:
Ref DB vehicles,
compounds that
do not cause
hepatotoxicity

Hepatitis Model Results

Felbamate Predictions



**Ref DB behavior
(1000's of samples)**

Above threshold:
Ref DB
compounds that
cause
hepatotoxicity

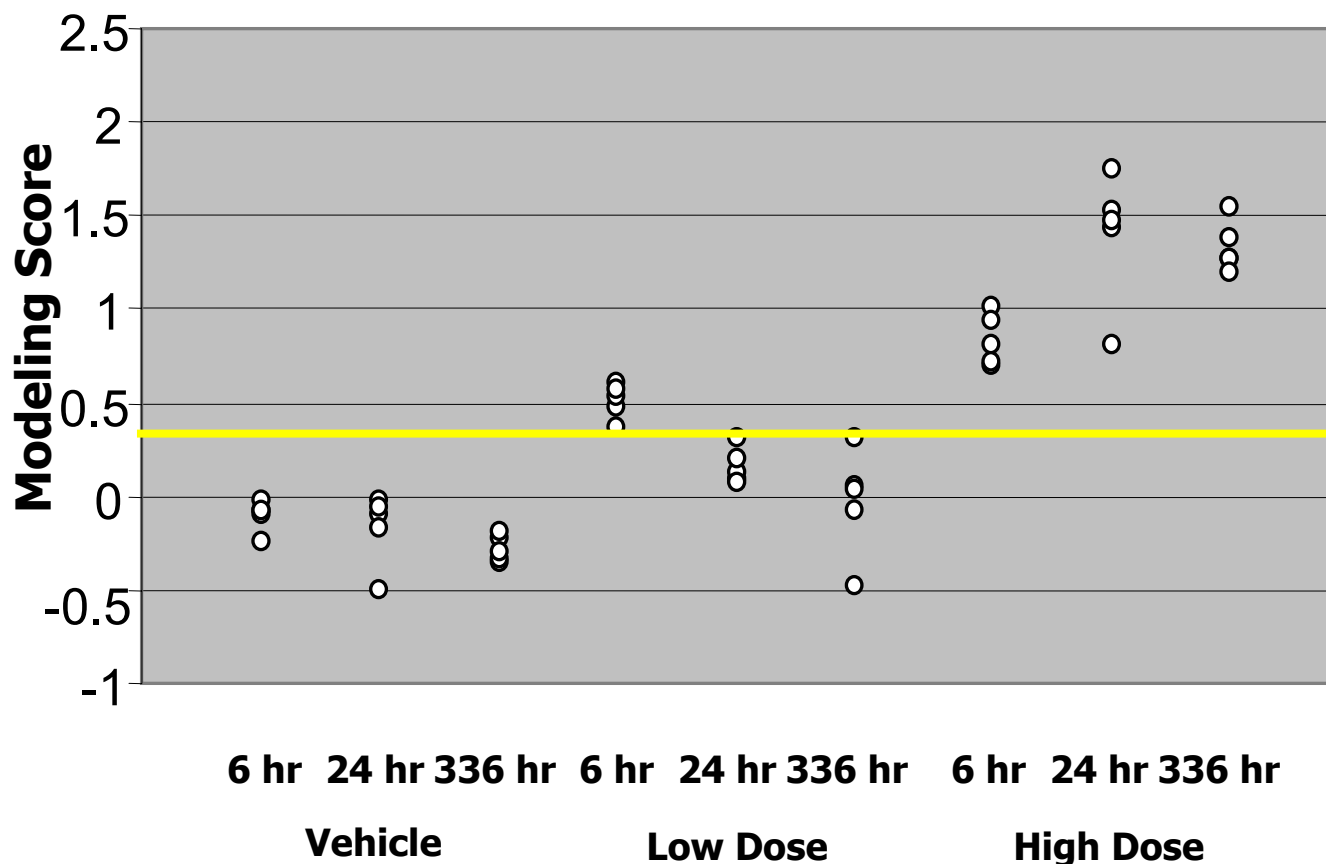
**Validated
Threshold**

Below threshold:
Ref DB vehicles,
compounds that
do not cause
hepatotoxicity

Inducer/Liver Enlargement Model Results

Ref DB behavior
(1000's of samples)

Felbamate Predictions

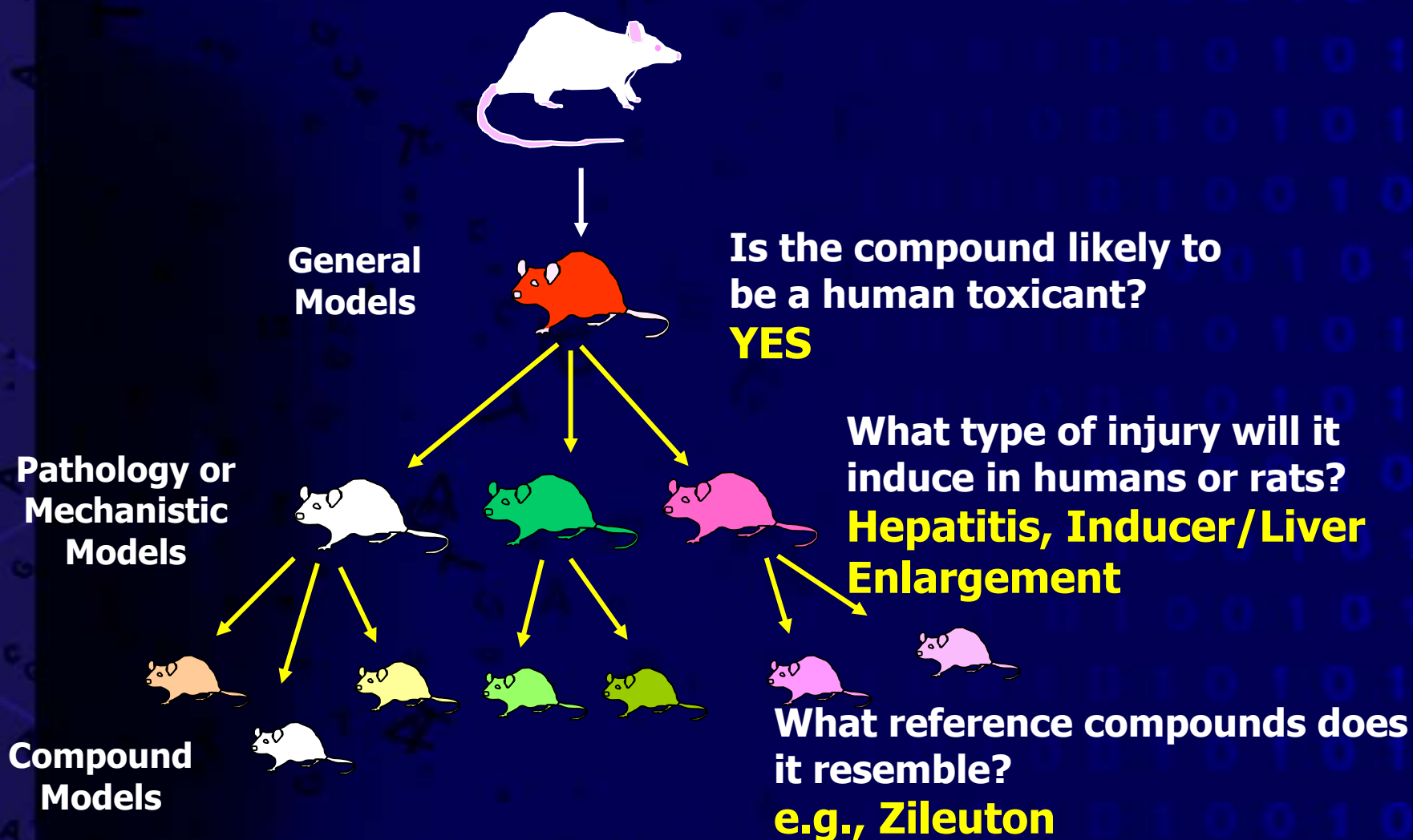


Above threshold:
Ref DB
compounds that
cause
hepatotoxicity

**Validated
Threshold**

Below threshold:
Ref DB vehicles,
compounds that
do not cause
hepatotoxicity

Felbamate: Human-Specific Hepatotoxicant



Felbamate: Summary

- **Preclinical testing failed to detect Felbamate as a potential hepatotoxicant**
- **Predictive toxicogenomic modeling and mechanistic assessment reveals this to be a potential hepatotoxicant**

Summary

- **Rigorous statistical cross-validation of liver models correctly reflect accuracy when presented with data generated from customer sites using novel compounds**
- **To date, customers have utilized our predictive models with >340 compounds**
 - **Of these, some were identified by our customers as human-specific hepatotoxicants and were correctly identified by our models**